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藏玄参中的抑精三萜皂甙

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摘要 从藏药植物藏玄参（*Oreosolen wattii* Hook. f.）的全草中分离到 4 个三萜皂甙，密蒙花甙（mimengoside）A 和 B 及醉鱼草甙（buddlejasaponin）I 和 Ia，另外还得到阿克甙（acteoside）和 6-羟基木犀草素-7-O-葡萄糖甙（6-hydroxyluteolin-7-O-glucoside）。体外抑精实验表明，4 个三萜皂甙均有较强的抑精活性，其中密蒙花甙 A 和醉鱼草甙 I 的活性高于阳性对照壬苯醇醚（NP-9）。

关键词 藏玄参，玄参科，三萜皂甙，抑精活性

SPERMICIDAL SAPONINS FROM OREOSOLEN WATTII

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Abstract Four triterpenoid saponins, mimengosides A and B and buddlejasaponins I and Ia were isolated from a traditional Tibetan medicinal plant, *Oreosolen wattii* Hook. f., together with acteoside and 6-hydroxyluteolin-7-O-glucoside. The *in vitro* spermicidal activities of the four saponins were tested by a modified Sander-Cramer method. Mimengoside A and buddlejasaponin I Showed stronger activity than the positive control NP-9.

Key words *Oreosolen wattii*, Scrophulariaceae, Triterpenoid saponin, Spermicidal activity

Oreosolen wattii Hook. f. is a small Scrophulariaceous herb native to high altitude mountain area at 4500～5000 m in Himalayan region. It is used in traditional Tibetan medicine for the treatment of fractures, open wounds, sprains and grasserie (Yang *et al.*, 1989). As part of our studies on the chemistry of medicinal plants in southwestern China, we undertook the chemical investigation of this plant. From water-soluble portion of the methanol extracts of whole herbs, six compounds (1～6) were obtained by repeat column chromatographies. By comparing with reported spectral data, four saikosaponin analogues were identified as mimengoside A (1) (Yamamoto *et al.*, 1993; Klimek *et al.*, 1992; Calis *et al.*, 1991), mimengoside B (2) (Klimek *et al.*, 1992; Ding *et al.*, 1992), buddlejasaponin I (3) (Yamamoto *et al.*, 1993, 1991) and buddlejasaponin Ia (4) (Yamamoto *et al.*, 1993). Two phenol glycosides were identified as acteoside (5) (He *et al.*, 1992) and 6-hydroxyluteolin-7-O-glucoside (6) (Ahmed *et al.*, 1987; Ranganthan *et al.*, 1980).

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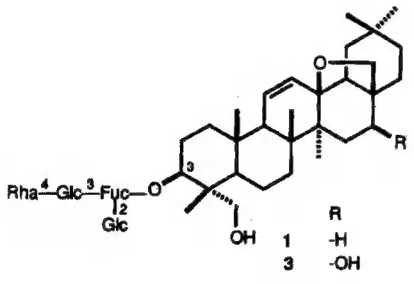
The direct *in vitro* spermicidal activities of saponins 1～4 were tested by a modified Sander-Cramer method, using different concentrations and comparing with nonoxynol (NP-9), a common used vaginal contraceptive drug. The results showed that all the saponins had sperm antimotility activity at a concentration of 1 mg / mL, and sperm decomposition in the case of 4 at 1 mg / mL was observed. The sequence of spermicidal activities from high to low was mimengoside A (1), buddlejasaponin I (3), NP-9, buddlejasaponin Ia (4) and mimengoside B (2). At the concentration of 0.01 mg / mL, saponin 1 had same effect with that of NP-9 at 0.1 mg / mL. Saponin 1 and 3 possessed an epoxy group between C-13 and C-28 positions. This may be the reason for their high activities. Compared to 1, saponin 3 which possessed an additional hydroxyl group at C-16 position was less active than 1. Similarity was observed for saponins 2 and 4. It is worthwhile to note that mimengoside A(1) and buddlejasaponin I(3) have stronger activity than the positive control NP-9.



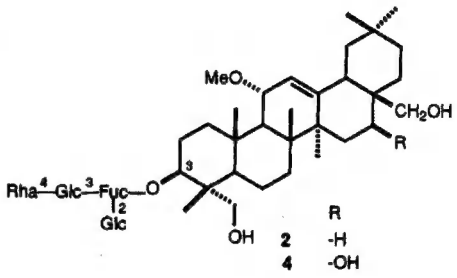


Table 1 Spermicidal activity of saponins 1～4 on human spermatozoa

Compound	Concentration (mg / mL)	Motility (%)
1	1	0
	0.10	0
	0.05	23
	0.01	36
2	1	20
3	1	0
	0.10	5
4	1*	0
	0.50	5
	0.10	100
NP-9 (positive control)	1	0
	0.50	0
	0.25	20
	0.10	39
PS (blank control)	–	>80

* Broken sperms was observed.

EXPERIMENTAL

Mps are uncorrected. ^{13}C and ^{1}H NMR spectra were measured on a Brucker AM-400 spectrometer using TMS as an internal standard. FAB-MS spectra were measured with VG AutoSpect mass spectrometer. CC was carried out with silica gel H, Sephedex LH-20, and Lichroprep RP-8, RP-18 (40～63 m, Merk), and TLC was conducted on a precoated Kieselgsel 60 F_{254} plate (0.2 mm, Merk) spraying by 10%

H_2SO_4 followed by heating.

Plant material The whole herbs of *Oreosolen wattii* were collected in mountain area of Zhongdian, Yunnan Province and identified by Prof. Yang Jingsheng. A voucher specimens is deposited in the herbarium of our institute.

Extraction and isolation Dried whole herbs (1.1 kg) were refluxed with MeOH. After removal of the solvent *in vacuo*, the black syrup extract (185.2 g) was suspended in H_2O and extracted with petrol, EtOAc and n-Bu OH successively.

The n-BuOH extract (53 g) was chromatographed on resin D-101 with H_2O, aq. MeOH and MeOH successively to yield eight fractions. Fr. 1 was repeatedly chromatographed on MCI gel (30% MeOH), Sephadex LH-20 (25% MeOH) and silica gel ($CHCl_3$-MeOH-H_2O = 3 ∶ 1 ∶ 0.1) to yield 5 (21 mg) and 6 (143 mg). Fr. 6 was chromatogrphed on silica gel ($CHCl_3$-MeOH-H_2O = 3 ∶ 1 ∶ 0.1), then on RP-8 (60% MeOH) to yield 3 (110 mg) and 4 (73mg). Fr. 8 was recrystallized in MeOH to yield (670 mg). The mother liquid together with Fr. 7 were repeatedly chromatographed on silica gel ($CHCl_3$-MeOH-H_2O = 3 ∶ 1 ∶ 0.1), Rp-18 (70% MeOH) and RP-8 (65% MeOH) to yield 1 (130mg) and 2 (800 mg).

Mimengoside A (1) Needles (MeOH), mp 243℃ ,$[\alpha]D^{25}$+16.3 ° (pyridine, C = 1.22). FAB-MS m / z: 1072$[M]^+$. ^1H NMR (pyridine-d_5): δ0.81 (3H, s, H-30), 0.89 (3H, s, H-29), 0.96 (6H, s, H-25, 26), 1.05 (3H, s, H-24), 1.32 (3H, s, H-27), 2.23 (1H, br d, J = 10Hz, H-2), 3.31 (1H, d, J = 6Hz, H-28), 3.71 (1H, d, J = 6Hz, H-28), 3.92 (1H, t, J = 8Hz, Glc′-2), 4.89 (1H, d, J = 8Hz, Fuc-1), 5.22 (1H, d, J = 8Hz, Glc′-1), 5.53 (1H, m, H-12), 5.56 (1H, d, J = 8Hz, Glc-1), 5.80 (1H, s, Rha-1), 5.94 (1H, d, J = 10Hz, H-12). ^{13}C NMR (pyridine-d_5): δ38.8 (1), 26.1 (2), 82.8 (3), 43.9 (4), 47.9 (5), 17.3 (6), 31.1 (7), 41.8 (8), 53.8 (9), 36.4 (10), 132.1 (11), 131.1 (12), 85.0 (13), 44.2 (14), 31.1 (15), 25.7 (16), 41.8 (17), 51.6 (18), 37.4 (19), 31.8 (20), 31.6 (21), 25.9 (22), 64.7 (23), 13.4 (24), 18.6 (25), 19.7 (26), 19.9 (27), 77.1 (28), 33.7 (29), 23.3 (30), Fuc 104.1 (1), 77.2 (2), 85.0 (3), 72.3 (4), 70.6 (5), 17.3 (6), Glc 105.0 (1), 75.6 (2), 78.9 (3), 72.1 (4), 77.1 (5), 61.4 (6), Glc′ 104.0 (1) 76.3 (2), 77.6 (3), 78.5 (4), 76.5 (5), 63.2 (6), Rha 102.8 (1), 72.8 (2), 72.6 (3), 74.0 (4), 70.5 (5), 18.7 (6). Acid hydrolysis of 1 (lmg) in 5% HCl-dioxane (1:1) at 100℃ for 2 hours afforded glucose, xylose, fucose and rhamnose which were dectected with TLC.

Mimengoside B (2) Needles (MeOH), mp 245～246℃ , $[\alpha]D^{25}$-7.4 ° (pyridine, C = 0.74). FAB-MS m / z: 1127$[M+Na]^+$. ^1H NMR (pyridine-d_5): δ0.88 (3H, s, H-30), 0.95 (3H, s, H-29), 0.97 (3H, s, H-25), 1.09 (3H, s, H-24), 1.15 (3H, s, H-27), 1.31 (3H, s, H-26), 3.21 (3H, s, OMe), 4.92 (1H, d, J = 8Hz, Fuc-1), 5.21 (1H, d, J = 8Hz, Glc′-1), 5.47 (1H, d, J = 3.2 Hz, H-12), 5.56 (1H, d, J = 8Hz, Glc-1), 5.78 (1H, s, Rha-1). ^{13}C NMR (pyridine-d_5): δ40.2 (1), 26.4 (2), 83.1 (3), 44.1 (4), 48.3 (5), 18.6 (6), 31.8 (7), 42.2 (8), 52.8 (9), 37.6 (10), 76.2 (11), 122.6 (12), 44.1 (13), 33.3 (15), 22.9 (16), 38.3 (17), 42.5 (18), 47.1 (19), 31.4 (20), 31.4 (21), 26.5 (22), 65.5 (23), 13.4 (24), 17.9 (25), 18.6 (26), 25.4 (27), 68.9 (28), 33.5 (29), 24.0 (30), 54.1 (OMe), Fuc 104.2 (1), 77.2 (2), 85.1 (3), 72.3 (4), 70.6 (5), 17.3 (6), Glc 105.1 (1), 75.6 (2), 78.9 (3), 72.2 (4), 77.0 (5), 61.5 (6), Glc′ 104.0 (1), 76.2 (2), 77.8 (3), 78.6 (4), 76.6 (5), 63.3 (6), Rha 102.9 (1), 72.8 (2), 72.6 (3), 74.0 (4), 70.5 (5), 18.6 (6).

Buddlejasaponin I (3) Amorphous white powder.$[\alpha]D^{25}$+13.2 ° (pyridine, C = 1.97). FAB-MS m / z: 1111$[M+Na]^+$. ^1H NMR (pyridine-d_5): δ0.91 (3H, s, H-30), 0.95 (3H, s, H-29), 0.97 (3H, s, H-29), 1.03 (3H, s, H-24), 1.09 (3H, s, H-27), 1.38 (3H, s, H-26), 3.32 (1H, d, J = 7Hz, H-28), 3.67 (1H, d, J = 10Hz, H-23), 5.66 (1H, d, J = 10Hz, H-12), 5.97 (1H, d, J = 10Hz, H-11), 4.89 (1H, d, J = 8Hz, Fuc-1), 5.51 (1H, d, J = 8Hz, Glc-1), 5.19 (1H, d, J = 8Hz, Glc′-1), 5.73 (1H, s, Rha-1). ^{13}C NMR (pyridine-d_5): δ38.8 (1),

25.9 (2), 82.8 (3), 43.9 (4), 48.0 (5), 17.6 (6), 31.7 (7), 42.4 (8), 53.2 (9), 36.4 (10), 131.2 (11), 132.1 (12), 84.1 (13), 45.8 (14), 36.2 (15), 64.3 (16), 47.1 (17), 52.3 (18), 38.0 (19), 31.7 (20), 34.9 (21), 26.2 (22), 64.7 (23), 12.8 (24), 18.6 (25), 20.2 (26), 21.0 (27), 73.2 (28), 33.8 (29), 24.0 (30), Fuc 104.2 (1), 77.2 (2), 85.0 (3), 72.3 (4), 70.6 (5), 17.3 (6), Glc 105.1 (1), 75.6 (2), 78.9 (3), 72.2 (4), 77.2 (5), 62.4 (6), Glc′ 104.0 (1), 76.3 (2), 77.6 (3), 78.6 (4), 76.6 (5), 63.3 (6), Rha 102.8 (1), 72.8 (2), 72.6 (3), 74.0 (4), 70.5 (5), 18.8 (6).

Buddlejasaponin Ia (4) Powder (MeOH).[α] D^{25}−13.2 ° (pyridine, C=0.61). FAB−MS m / z: 1143[M+Na]$^+$. ^{1}H NMR (pyridine−d_5): δ0.89 (3H, s, H−30), 0.98 (3H, s, H−29), 1.07 (3H, s, H−25), 1.09 (3H, s, H−24), 1.10 (3H, s, H−27), 1.38 (3H, s, H−26), 1.39 (3H, d, J=8Hz, Fuc−6), 1.67 (3H, d, J=8Hz, Rha−6), 1.93 (1H, d, J=4Hz, H−9), 3.21 (3H, s, OMe), 3.71 (1H, d, J=12Hz, H−23), 3.81 (1H, dd, J=8, 3Hz, H−11), 4.08 (1H, dd, J=12, 5Hz, H−3), 4.90 (1H, d, J=8Hz, Fuc−1), 5.20 (1H, d, J=8Hz, Glc′−1), 5.52 (1H, d, J=3Hz, H−12), 5.56 (1H, d, J=8Hz, Glc−1), 5.81 (1H, s, Rha−1). ^{13}C NMR (pyridine−d_5): δ 40.2 (1), 26.1 (2), 82.9 (3), 44.0 (4), 48.3 (5), 18.5 (6), 33.4 (7), 41.1 (8), 54.1 (9), 38.2 (10), 76.1 (11), 122.8 (12), 148.3 (13), 44.0 (14), 36.9 (15), 66.5 (16), 38.2 (17), 43.8 (18), 47.2 (19), 31.2 (20), 33.4 (21), 26.5 (22), 65.1 (23), 13.3 (24), 17.9 (25), 18.6 (26), 26.4 (27), 68.8 (28), 33.4 (29), 26.5 (30), 54.1 (OMe), Fuc 104.0 (1), 77.2 (2), 85.0 (3), 72.3 (4), 70.6 (5), 17.2 (6), Glc 105.0 (1), 75.6 (2), 78.9 (3), 72.1 (4), 77.1 (5), 61.5 (6), Glc; 104.0 (1), 76.3 (2), 77.8 (3), 78.6 (4), 76.5 (5), 63.2 (6), Rha 102.8 (1), 72.8 (2), 72.6 (3), 74.0 (4), 70.5 (5), 18.6 (6).

Acteoside (5) ^{1}H NMR (CD_3OD): δ aglycone 6.70 (1H, d, J=2.0Hz, H−2), 6.68 (1H, d, J=8Hz, H−5), 6.56 (1H, dd, J=8, 2Hz, H−6), 2.78 (1H, m, H−7), caffeoyl 7.05 (1H, d, J=2Hz, H−2), 6.78 (1H, d, J=8Hz, H−5), 6.94 (1H, dd, J=8, 2Hz, H−6), 7.59 (1H, d, J=16Hz, H−7), 6.28 (1H, d, J=16Hz, H−8). ^{13}C NMR (CD_3OD): δ aglycone 131.5 (1), 116.5 (2), 114.4 (3), 145.9 (4), 117.1 (5), 121.3 (6), 36.3 (7), 72.0 (8). caffeoyl 127.6 (1), 115.3 (2), 146.5 (3), 149.5 (4), 116.3 (5), 123.1 (6), 147.9 (7), 114.7 (8), 168.3 (C=O). glc 104.0 (1), 75.8 (2), 81.5 (3), 70.5 (4), 76.0 (5), 62.3 (6). rha 103.0 (1), 72.0 (2), 72.2 (3), 73.7 (4), 70.5 (5), 18.3 (6).

6−Hydroxyluteolin−7−O−glucoside (6) Brown powder, FAB−MS m / z: 465[M+1]$^+$, 303 [M−Glu]$^+$. ^{1}H NMR (DMSO−d_6):δ6.65 (1H, s, H−3), 6.95 (1H, s, H−8), 7.39 (1H, d, J=2Hz, H−2′), 6.92 (1H, d, J=8Hz, H−5′), 7.41 (1H, dd, J=8, 2Hz, H−6′), 5.00 (1H, d, J=8Hz, G−1). ^{13}C NMR (DMSO−d_6): δ aglycone 164.3 (2), 102.5 (3), 182.2 (4), 149.0 (5), 130.5 (6), 151.3 (7), 94.1 (8), 146.6 (9), 105.9 (10), 121.7 (1′), 113.5 (2′), 145.7 (3′), 149.7 (4′), 116.0 (5′), 118.9 (6′). Glc: 101.1 (1), 73.3 (2), 75.9 (3), 69.8 (4), 77.3 (5), 60.7 (6). Acid hydrolysis of 6 (lmg) in 5% HCl−dioxane (1:1) at 100℃ for 2 hours afforded glucose which was identified with TLC.

Semen samples All semen samples were produced by masturbation into specimen containers after 5 days of prior sexual abstinence. Semen analysis was performed 30 min. after ejaculation using standard established procedures. All ejaculates used had a volume of >2ml, sperm concentration >40× 10^6 sperms / ml, motilities of >80% and normal morphology of >70%.

Spermicidal test According to a modified Sander−Cramer method, an aliquot of 0.5 μL liquefied semen was thoroughly mixed with 2.5 μL samples at different concentrations on a pre−incubated glass plate at 37℃. After covered, the mixtures were immediately examined under a low power microscope (100×) for sperm motilities. The percentage of motilities was counted under a high power microscope (400×). The test was performed triplicate with 3 different semen samples and each time PS control set was prepared (where the drugs were replaced by physiological saline).

The spermicidal activity of NP-9 was tested in the same way to confirm the reliability of the assay at the beginning, middle and ending period. A series of solutions of each saponin and NP-9 were prepared in physiological saline.

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